

If the distance between well centers is made 8 mm and the well diameter 4 mm, the reactants may be delivered in 3 doses of 0.01 ml at approximately 2-hour intervals. If the well geometry is increased to 10 or 13 mm to allow for a well diameter of 6 mm, the complete 0.03 ml of reactants may be delivered at one time.

When precipitation is complete the slide is washed in tap water to remove excess protein from the wells. Unprecipitated protein is eluted by soaking the slide in buffered saline for 24 hours, followed by 2 rinses of 1/2 hour each in distilled water. Slides are stained for 20-30 minutes in Crowle's triple stain (Immunodiffusion, 1961, Academic Press) or in dilute water-soluble nigrosin, destained in 1% acetic acid, and air dried to a film. The finished slide may be used directly in the photographic enlarger to prepare prints, and is in itself a convenient permanent record of the test.

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An improved technique for fecundity and hatchability tests.

A new technique of collecting eggs for fecundity or hatchability tests has been devised, which has the following advantages: homogeneous egg laying surface resulting in uniform egg distribution,

rapidly and easily dispensed medium, medium lacking extraneous food components (such as charcoal), transparent medium allowing scoring of burrowing larvae, and easily cleaned and reused equipment.

The medium consists of 1 g. Bacto-agar, 100 ml. water and 15 ml. white Karo syrup, which is dispensed with an automatic syringe while hot. This medium is then sprayed with a water suspension of bakers yeast immediately before use.

The equipment consists of two variations on the same theme. One variation supplies a black background to facilitate counting. The other presents a transparent background, which allows visual examination of eggs without the removal of the cap from the test bottle.

The test bottles are constructed from 40 dram Plastainer bottles (ca. 2" x 3 1/4") available from Owens-Illinois Glass Co., Toledo, Ohio, at a cost of about \$5 per carton (6 dozen). Extra caps are available at about \$20 per thousand. The screw caps are made of Teflon and the bottle of clear plastic. A hole is punched in the cap top with a die about 1 1/4" in diameter, and then a piece of plexiglass 1/16" thick is glued to the outer surface of the cap over the hole. The plexiglass may be either black or transparent, giving the two varieties of background. A critical factor in construction is the cement for glueing the cap and the plexiglass. The most satisfactory one tried was Eastman 910 adhesive, available from the Tennessee Eastman Company, Kingsport, Tennessee, at a cost of \$8 per bottle. One bottle is sufficient to glue about 400 caps. Also the surface of the cap must be roughened with hardware cloth or a file before glueing. The glue is spread in a very thin band completely around the hole in order to get a water-tight seal. Leaks may be sealed with a band of Duco cement around the external cap-plexiglass junction.

Counting is easily accomplished by marking the agar surface into regions with a blunt needle under about 40X magnification or less. Eggs or larvae may be conveniently transferred to food bottles by transferring agar and eggs or larvae with a small spatula (eg., No. 19240, Curtin Cat. 40) bent at a convenient angle to work inside the cap. Larvae may crawl off the agar surface, but for caps changed every 24 hours or so, it is not a serious problem. Empty egg cases are easily distinguished from unhatched eggs.

An additional advantage of this technique is the practicality of a permanent photographic record of the egg production or hatchability, especially since the eggs are well spread over the surface. The quickest technique using the transparent plexiglass caps in a "contact print" of the cap on photographic paper (available in bulk rolls about 4 1/4" wide) where the shadow of the egg is recorded. Enlargement prints are possible by placing the cap in the film plane of a darkroom enlarger. More detailed records may be made by microfilming the black plexiglass caps with a 35 mm. camera. Examination of the negative either in a microfilm reader or under a dissecting scope allows easy egg counts, hatch scores, or even some egg development scores. It appears counts could even be made by visual scanners in use by automatic data processing systems.